Assessing the Impact of Toxicants on the Microbiome

Food and Drug Administration
NCTR
National Center for Toxicological Research

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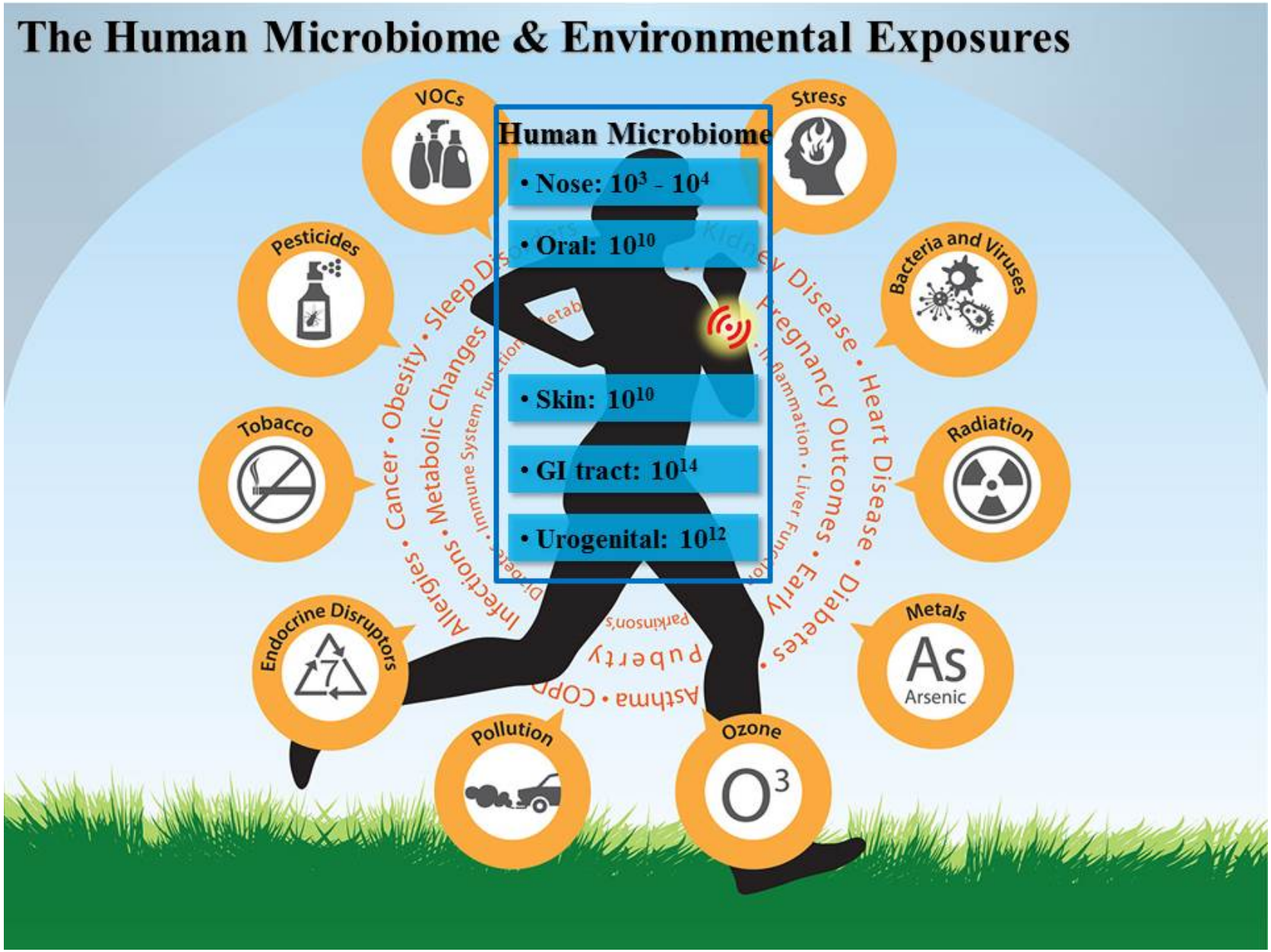
Micro & Macro views


The Human Microbiome & Environmental Exposures
Human Microbiome
• Nose: 10^3 - 10^4
• Oral: 10^{10}
• Skin: 10^{10}
• GI tract: 10^{14}
• Urogenital: 10^{12}
VOCs
Stress
Pesticides
Bacteria and Viruses
Tobacco
Radiation
Endocrine Disruptors
Metals
As
Arsenic
Pollution
Ozone
O^3
Cancer • Obesity • Sleep
Metabolic Changes
Infections
Allergies
Pregnancy Outcomes
Diabetes
Heart Disease
Asthma
Puberty
Parkinson's

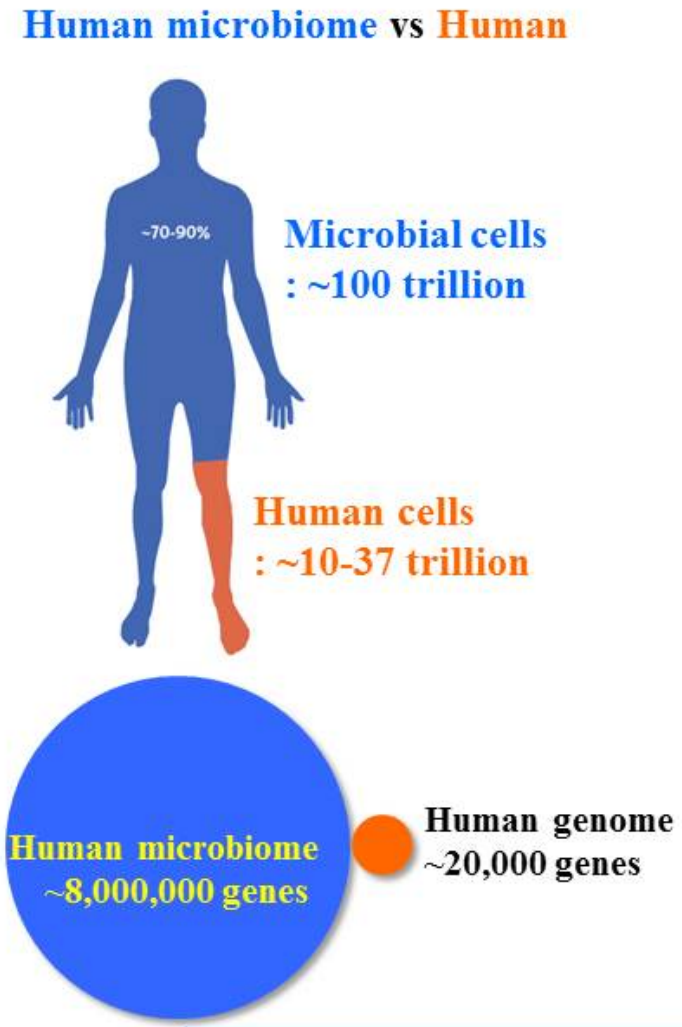
What is the Human Microbiome?

Ecological definition:

the ecosystem made up of microbes within and on the human body—that is, the collection of microbes (bacteria, archaea, fungi, viruses and single-cell eukaryotes) that live in the human "habitat".

Genetic definition:

the entire collection of genes found in all of the microbes associated with a particular host



Historical Perspective

1673: van Leeuwenhoek-First observation of live microorganisms

1857-1861: Pasteur-Fermentation and pasteurization

1876-1883: Koch-Germ theory of disease, Pure culture
Mycobacterium tuberculosis, *Vibrio cholerae*

1884: Metchnikoff-Probiotics

1886: Escherich-Enterobacter of infants

1928: Fleming-the first chemical compound with antibiotic properties, **Penicillin**

1946: Lederberg and Tatum-bacterial conjugation
2001: Joshua Lederberg first suggested the concept of the **Human microbiome**

1953: Watson and Crick-DNA structure

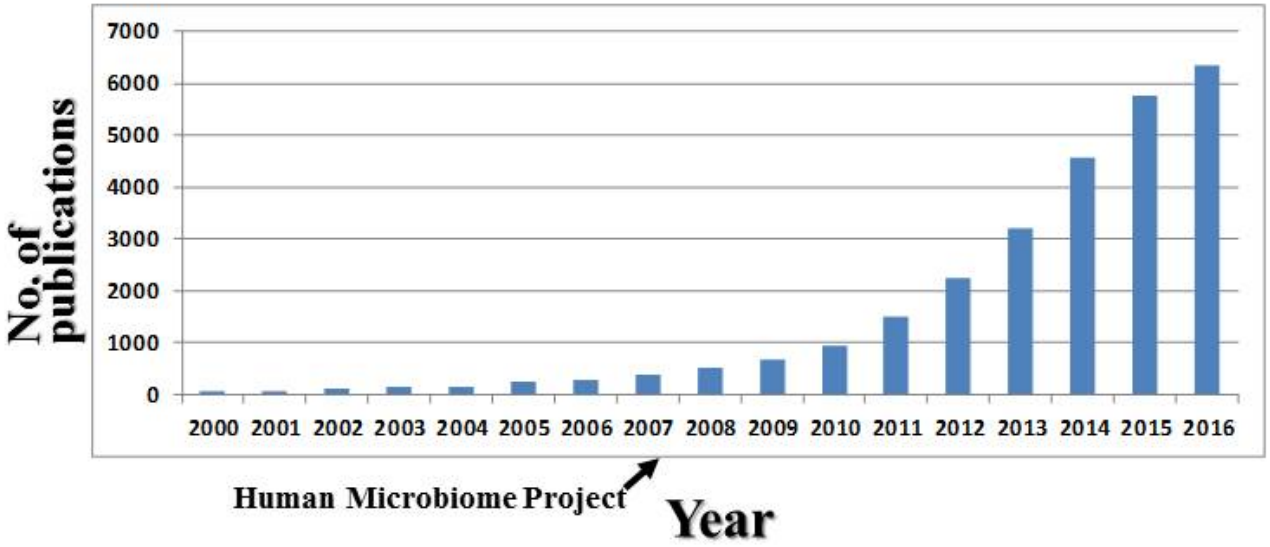
1977: Sanger-Sanger sequencing

1983: Mullis-PCR

1990-2003: Human Genome project

2007: Human Microbiome project

Microbiome studies

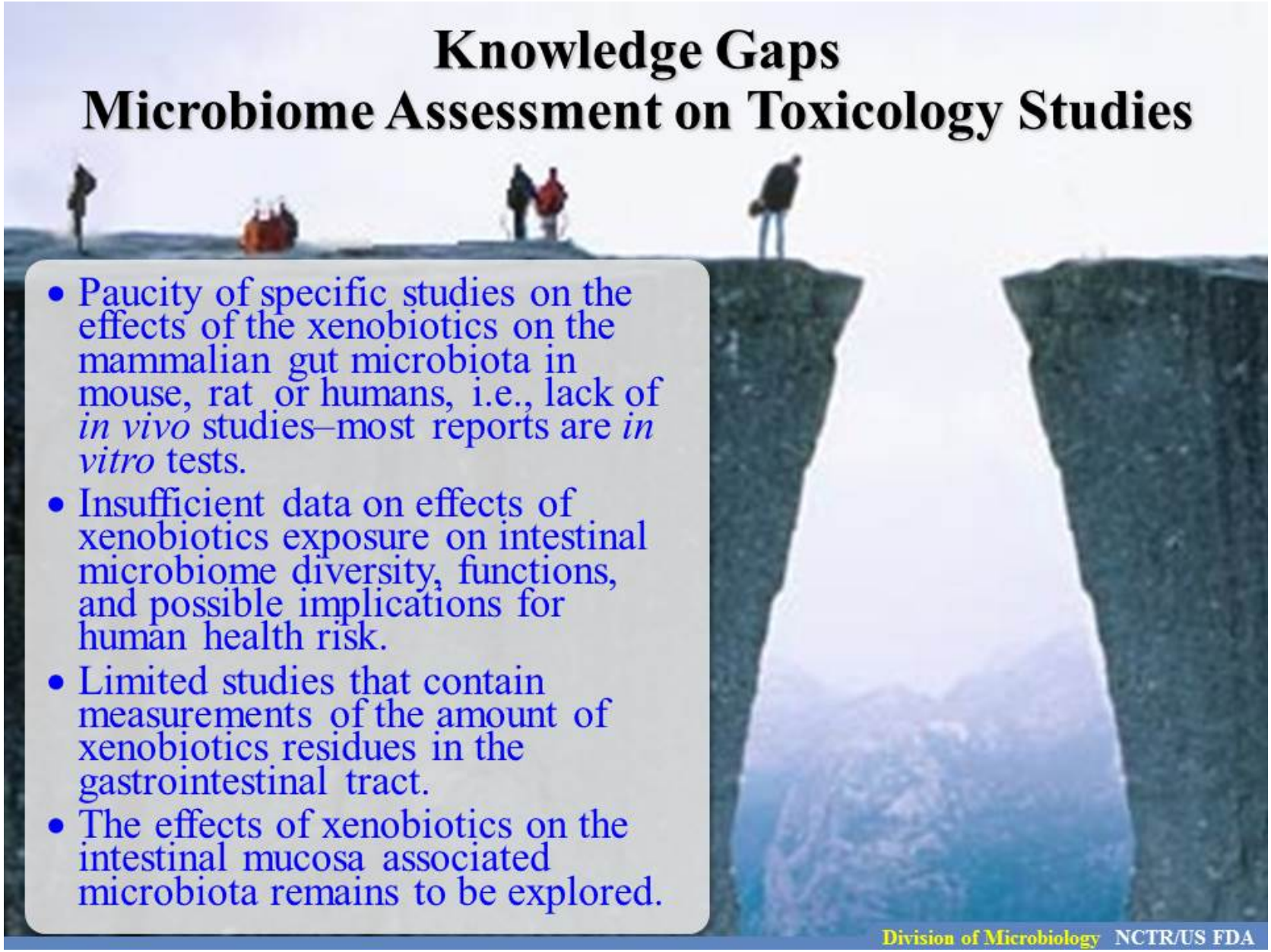


Microbiome

Microbiome research is a major growth area

- >23,400 new publications since 2007 (HMP).
- ~1,600 total prior to 2007.
- Applications for nutrition, drug and food safety, environmental health, precision medicine, etc.

Knowledge Gaps
Microbiome Assessment on Toxicology Studies



- Paucity of specific studies on the effects of the xenobiotics on the mammalian gut microbiota in mouse, rat or humans, i.e., lack of *in vivo* studies–most reports are *in vitro* tests.
- Insufficient data on effects of xenobiotics exposure on intestinal microbiome diversity, functions, and possible implications for human health risk.
- Limited studies that contain measurements of the amount of xenobiotics residues in the gastrointestinal tract.
- The effects of xenobiotics on the intestinal mucosa associated microbiota remains to be explored.

Assessment of the Role that the Microbiome May Play in the Toxicity of Xenobiotics

National Toxicology Program Capability Building for Microbiome Assessment on Toxicology Studies

PI: Carl E. Cerniglia, Ph.D. (Division of Microbiology)
Co-PIs: Paul Howard, Ph.D. (Office of Scientific Coordination)
Sangeeta Khare, Ph.D. (Division of Microbiology)

Dr. Vicki Sutherland NIEHS/NTP

Project Number: E0220101 7/9/2015

Specific goals

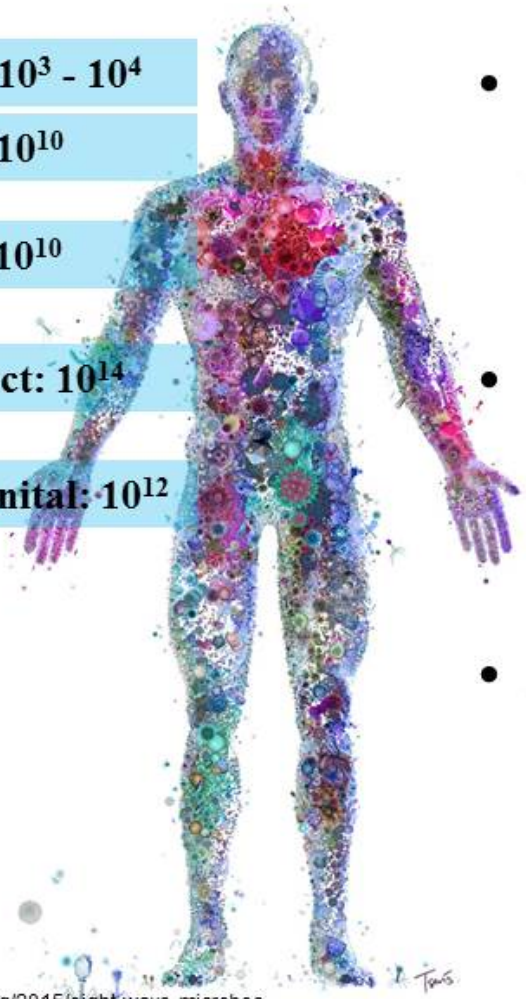
- To conduct host-microbiome assessments of NCTR/NIEHS/NTP studies to evaluate the impact on the gastrointestinal microbiome and immunity
- To establish a standardized approach within the NTP program for
  1) Sample collection and methodologies for gastrointestinal analysis
  2) Standardized data analysis and approaches for data-repository and data presentation
  3) Establishing science-based standards for conducting hazard analysis of FDA-regulated products and improving the prediction of the safety assessment for such products.

FDA/NCTR NTP Significance

The results will be a step towards NCTR/FDA and NTP readiness to evaluate innovative emerging technologies for improving product assessment and quality, as well as, modernize toxicology.

Where is the human microbiome located?

- Nose: 10^3 - 10^4
- Oral: 10^{10}
- Skin: 10^{10}
- GI tract: 10^{14}
- Urogenital: 10^{12}



http://whyfiles.org/2015/eight-ways-microbes-keep-you-healthy/

- Our human body sites are colonized by an enormous number of microorganisms, of which the majority is bacterial species, and they form complex communities called the human microbiota.
- The total number of these bacterial cells is estimated to be more than 10^{14}, accounting for 10 times more than the total number of eukaryotic cells that compose a human individual.
- Among them, the gut microbiota is the largest and most complex, and is composed of more than 1,000 different intestinal microbes.

Microbiome Analysis for Toxicology Risk Assessments

RNA, Protein, metabolite

Total DNA extraction

16S rRNA-based approach

- PCR amplification of 16S rDNA

Whole-genome shotgun approach

- NGS shotgun library preparation

NGS Sequencing

Phylogeny

- Bacterial composition & diversity

Metagenome

- High resolution microbiota profiling
- Gene contents from uncultivated microbes
- Genome sequences

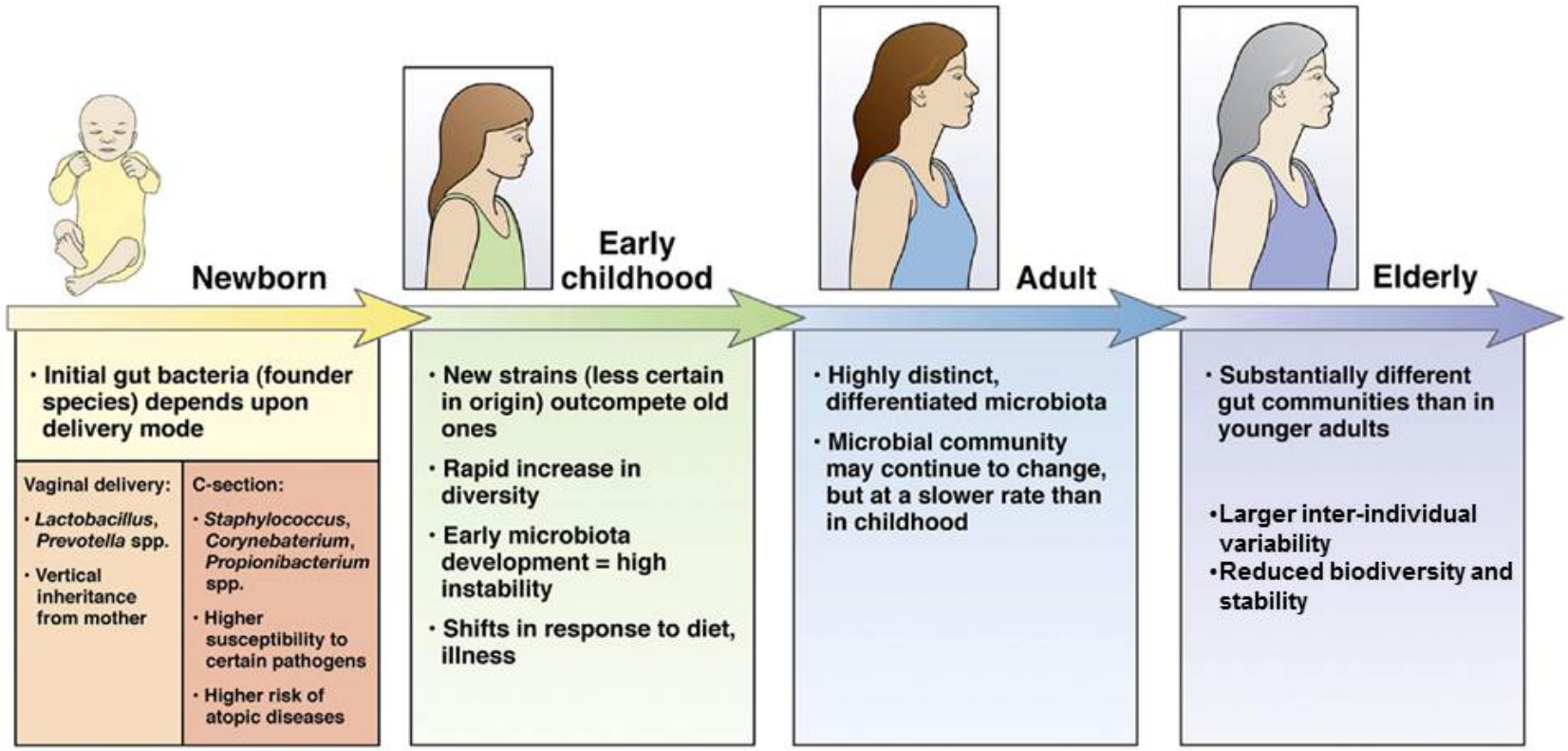
Metagenomics

Genomics

Bioinformatics

Meta-omics

Where does our microbiome come from? The first inoculum as an infant through continued change, modified by diet, genetics and the environment throughout life

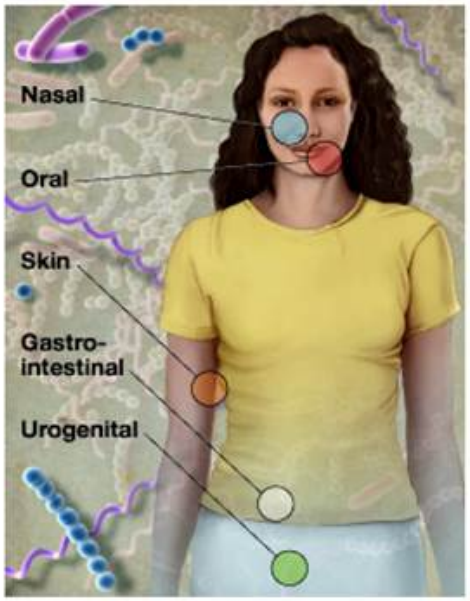


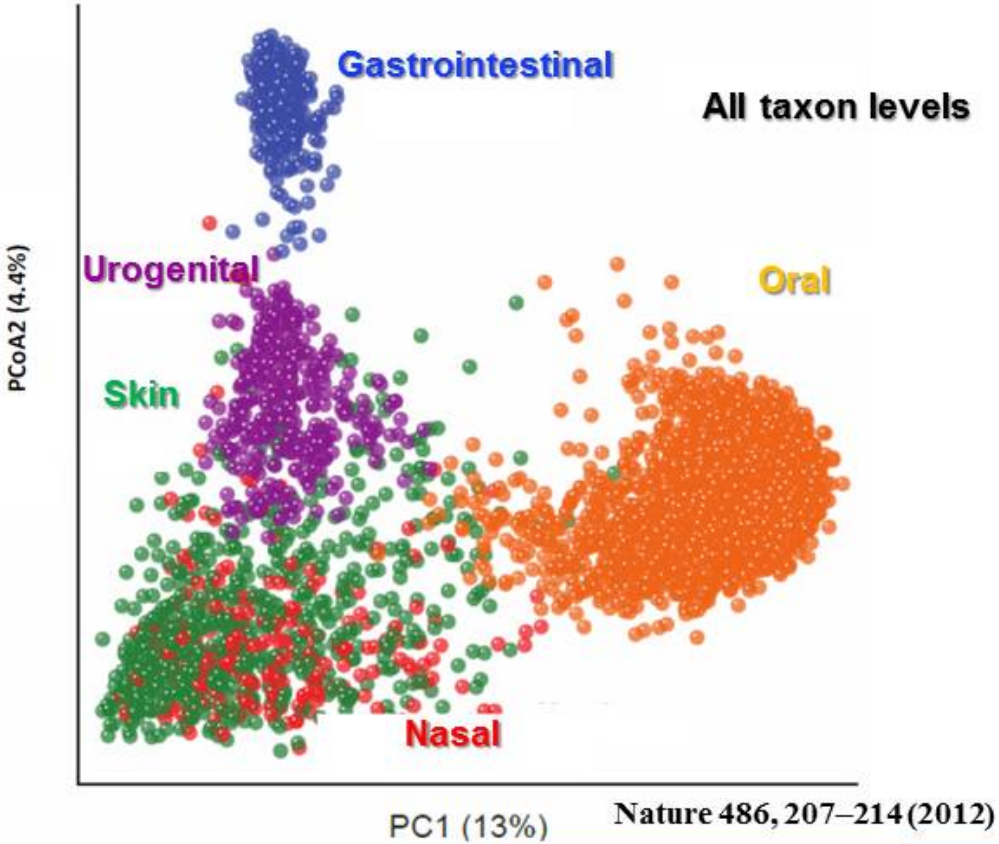
Dominguez-Bello, M.G. *et al.*, Gastroenterology (2011)

Age is an important determinant that impacts microbial composition of GI tract and also impacts toxicant absorption, bioavailability, and metabolism of xenobiotics.

Is everyone's microbiome the same?

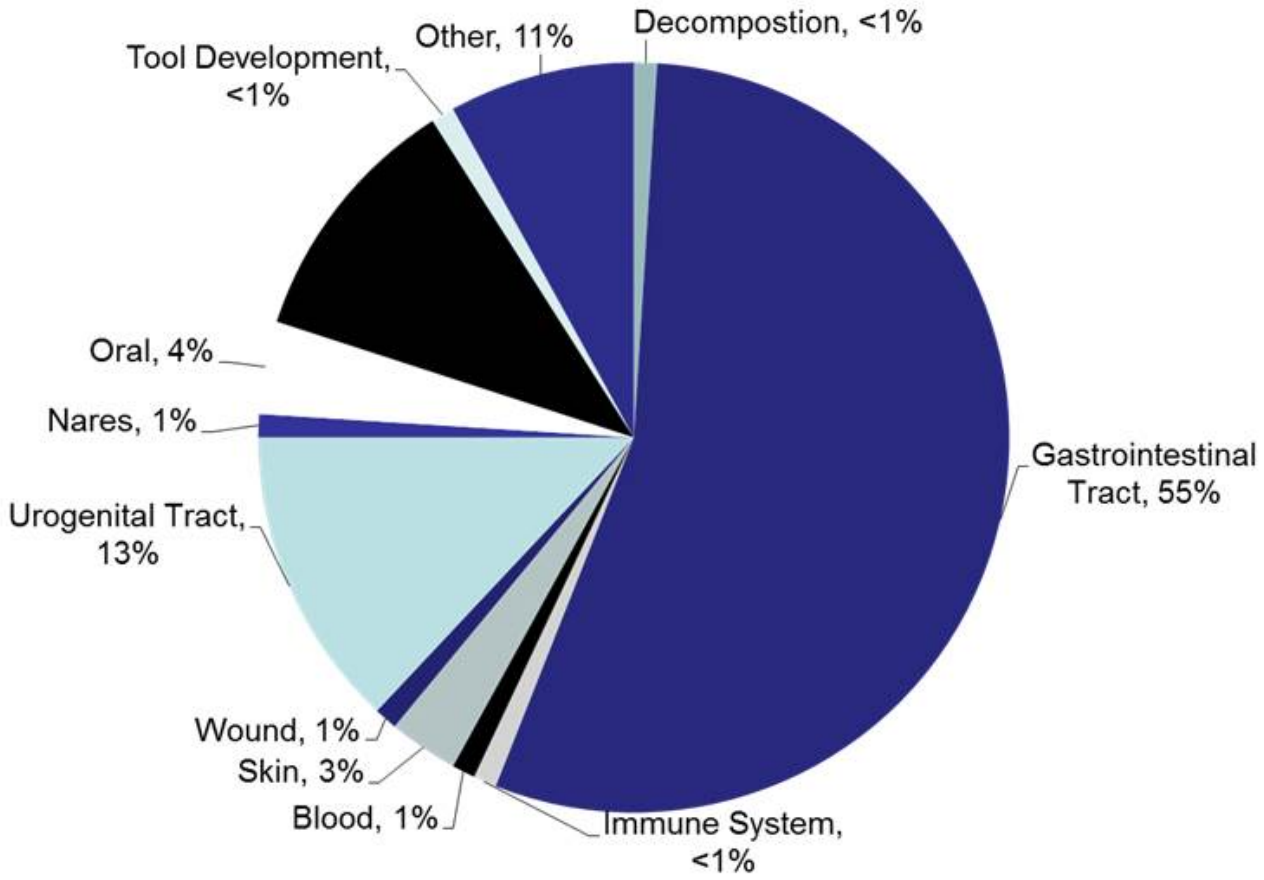
In adults, each part of the body supports a distinct microbial community



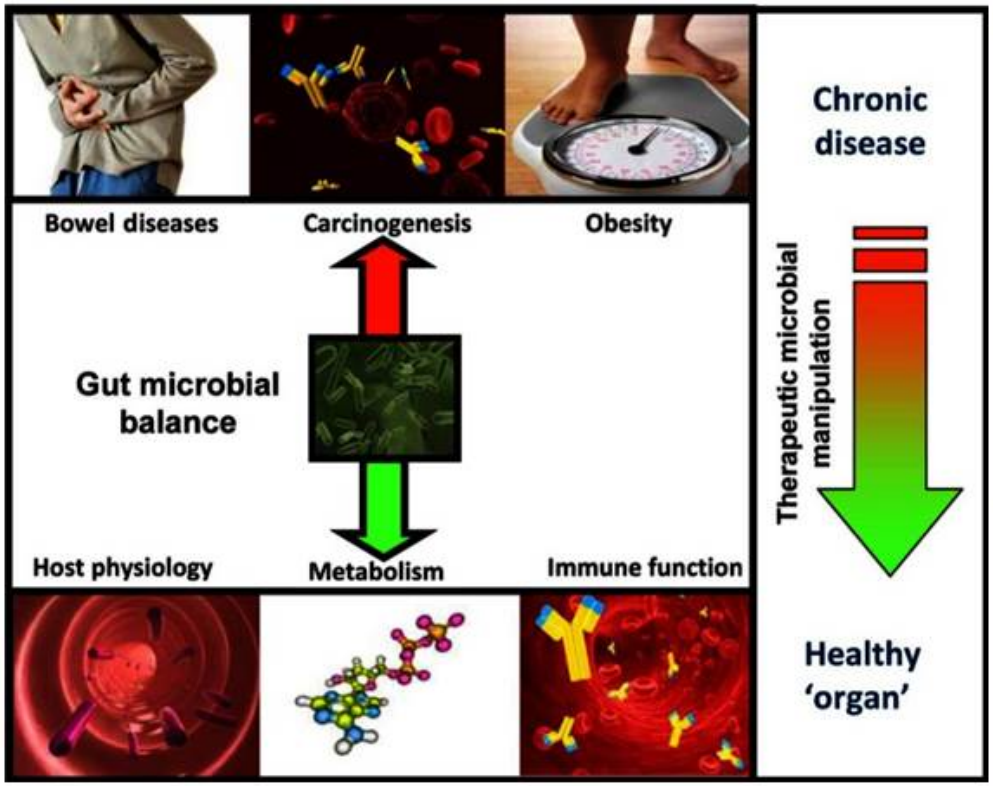


Nature 486, 207–214 (2012)

Human Microbiome Research Funding



What is the relationship between the gut microbiota in health and intestinal disease?

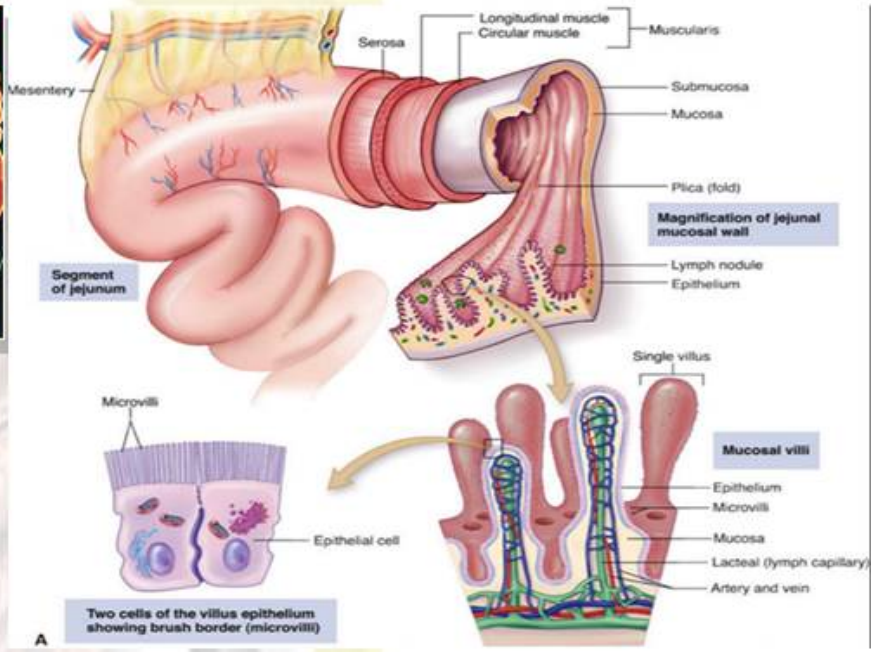


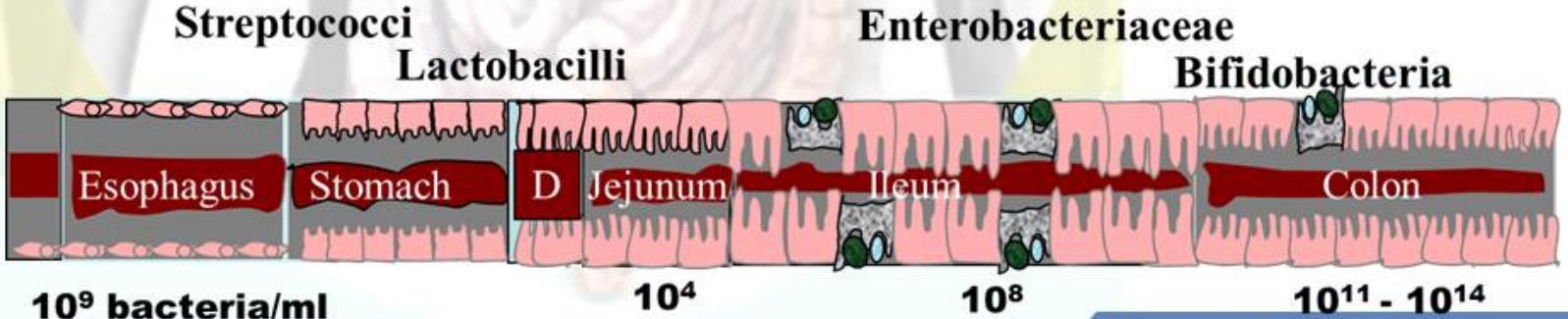
Guinane, CM and Cotter PD, Therap Adv Gastroenterol. 2013. 6(4):295-308

- The gastrointestinal microbiota play a role in host physiology, metabolism and nutrition.
- An alteration in the gut microbial community is linked to a number of intestinal conditions, including cancer, obesity, autism, depression, asthma, and a variety of bowel disorders.
- The contribution of beneficial components of the gut microbiome to host physiology, metabolism and immune function has become the focus of ever more attention, and will undoubtedly lead to new therapeutic approaches.

Host Influences in Gut Microbial Ecology

- Age
- Genetics
- Diet, Drugs
- Environmental
- Stress responses
- Defense mechanism
- Health status
- Newborn delivery mode





Which bacteria make up the gut microbiota?

The five dominant bacterial phyla (Firmicutes, Bacteriodetes, Actinobacteria, Proteobacteria, Verrucomicrobia) and one archaea (Methanobrevibacter)

Firmicutes
- **Clostridium**
- **Eubacterium**
- **Lactobacillus**

Bacteriodetes
- **Bacteriodes**
- **Prevotella**

Actinobacteria
- **Bifidobacterium**

Proteobacteria
- ***E. coli***
- **Desulfovibrio**

Verrucomicrobia
- **Akkermansia**

Archaea
- **Methanobrevibacter**

90%

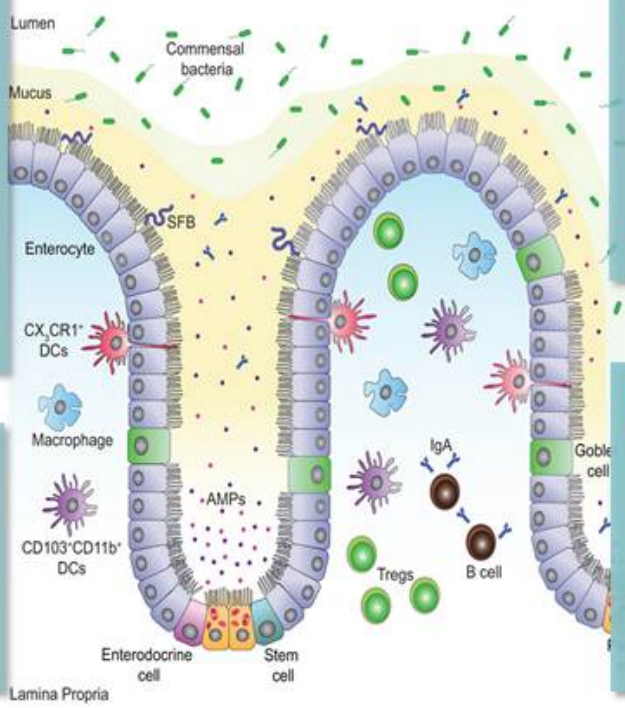
What is the intestinal microbiota doing as an essential component of human physiology?

Intestinal mucosa is the largest surface area in the human body

Potential Microbiological Endpoints in Toxicology Assessments

Structural Functions
- Barriers
- Apical tightening of tight junction
- Development of immune system
- Control Intraepithelial cell differentiation and proliferation

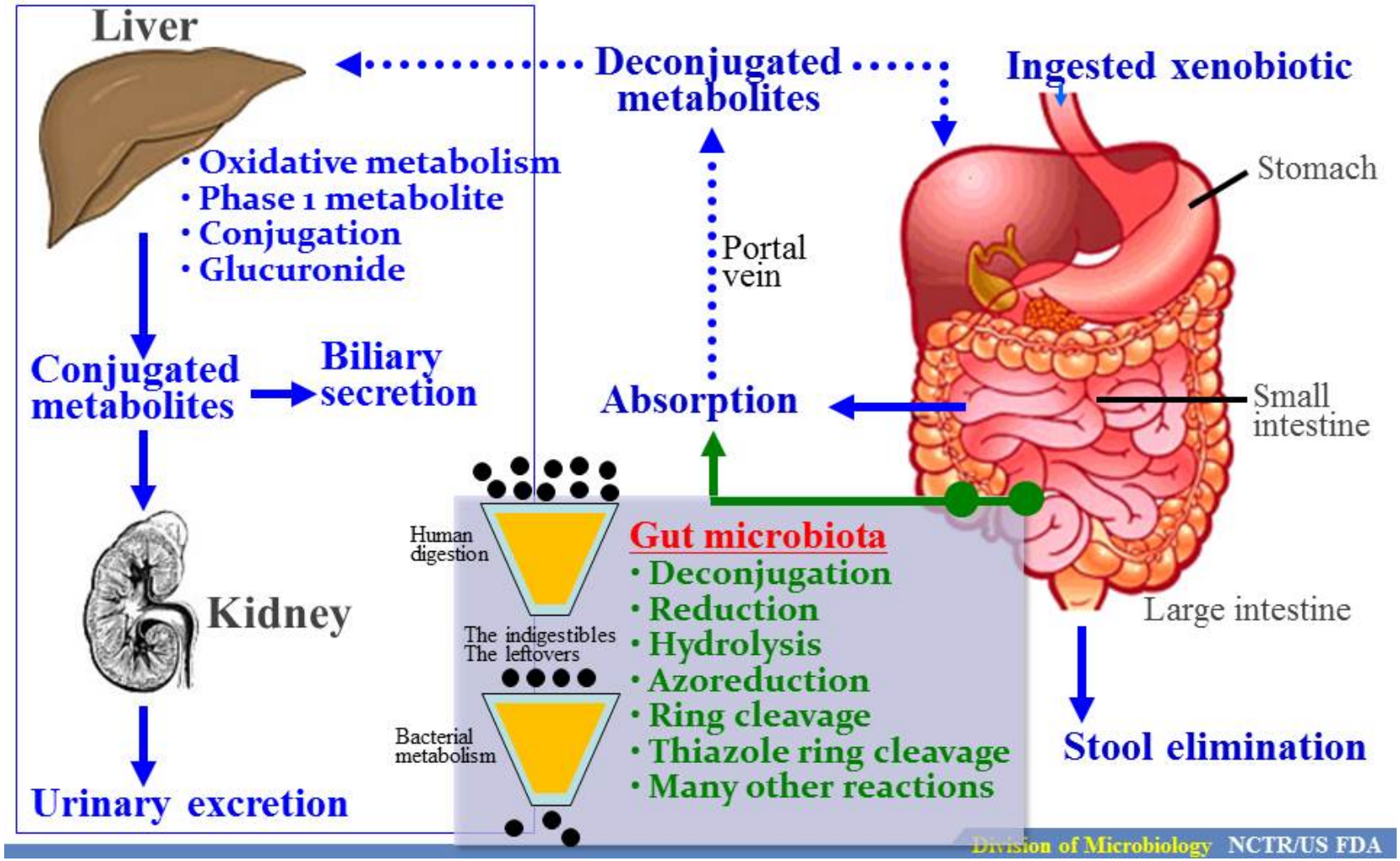
Immune Functions
- Peyer's patch-mucosal immunity hub
- Epithelial signaling
- Inflammatory responses



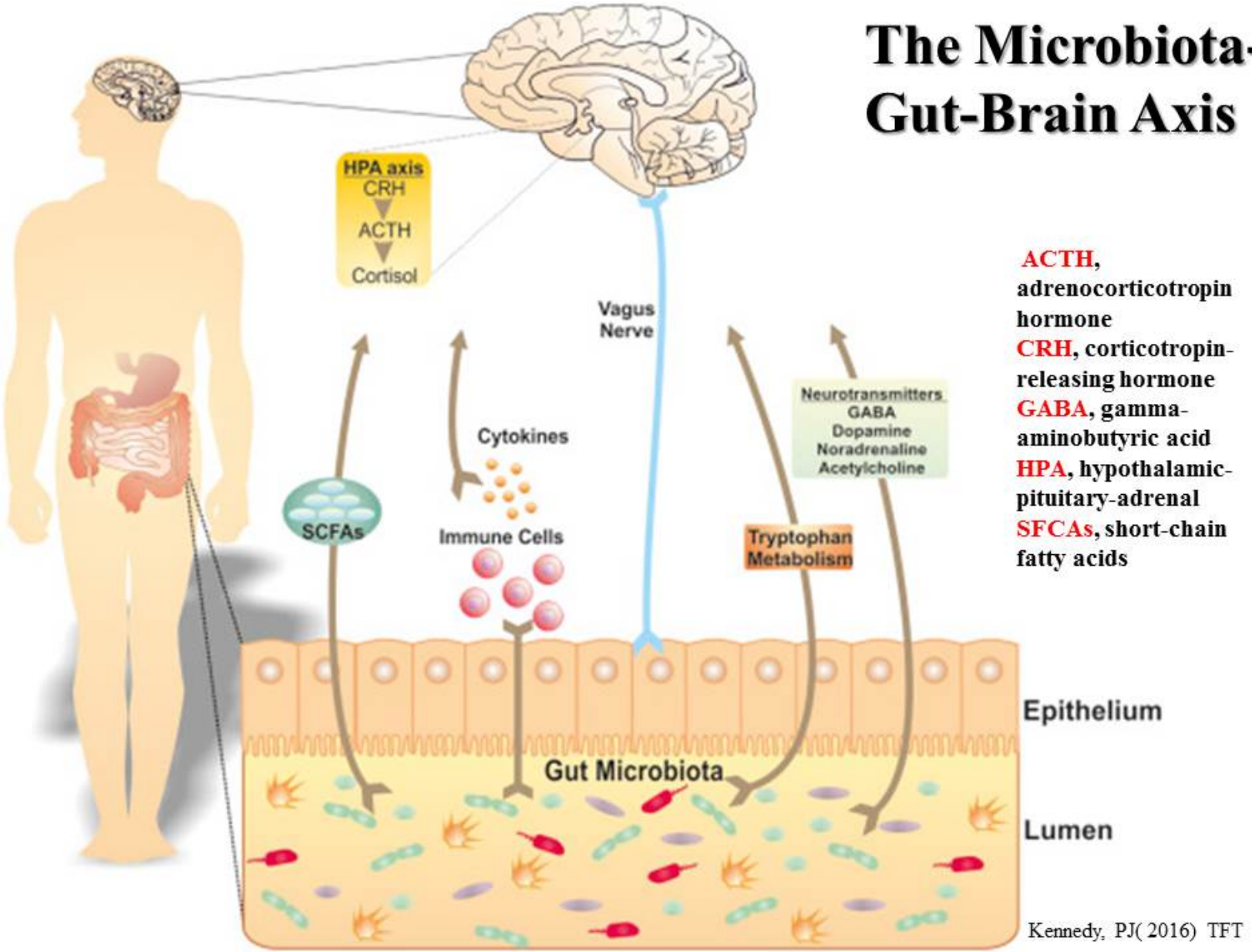
Defensive Functions
- Pathogen displacement
- Nutrient competition
- Receptor competition
- Production of antimicrobial factors
- Induction of IgA

Metabolic Functions
- Metabolize dietary carcinogens
- Synthesize biotin and folate
- Ferment non-digestible dietary residue and mucus

The metabolism of xenobiotics by human gut microbiota



The Microbiota-Gut-Brain Axis

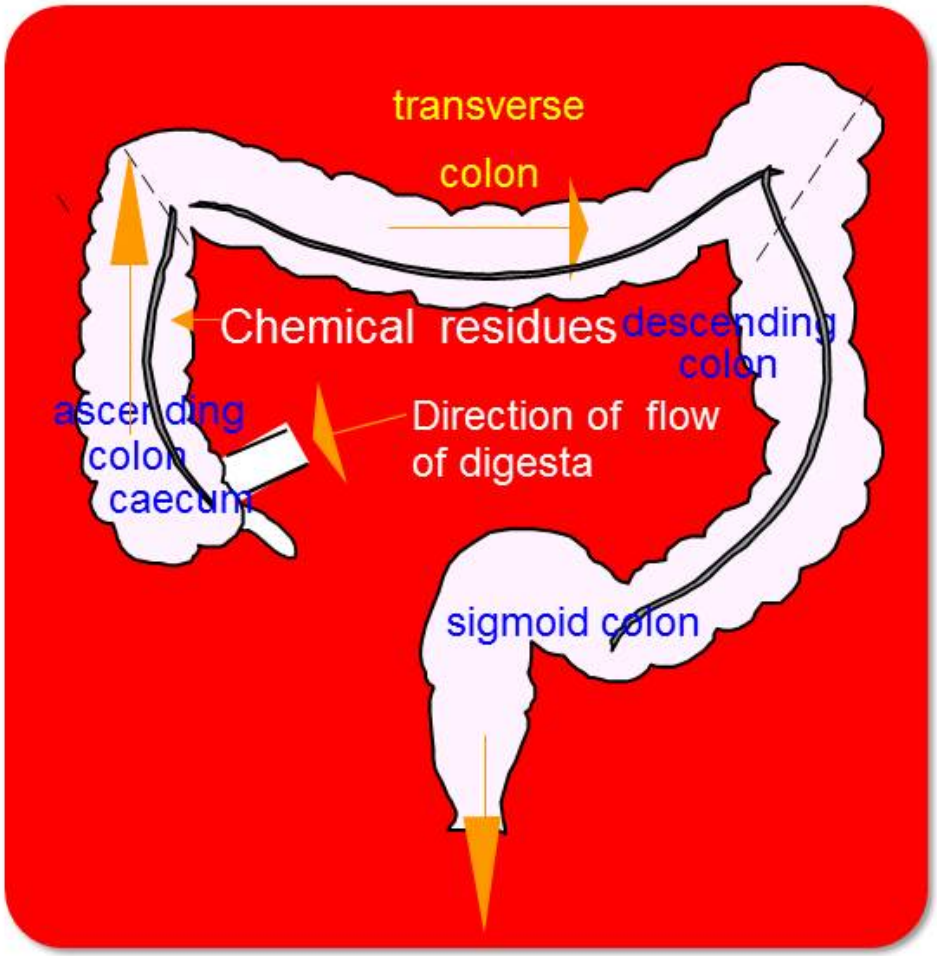


ACTH, adrenocorticotropin hormone
CRH, corticotropin-releasing hormone
GABA, gamma-aminobutyric acid
HPA, hypothalamic-pituitary-adrenal
SFCAs, short-chain fatty acids

Kennedy, PJ(2016) TFT

Exposure of Intestinal Bacteria to the Ingested Chemical Residues Under Different Scenarios

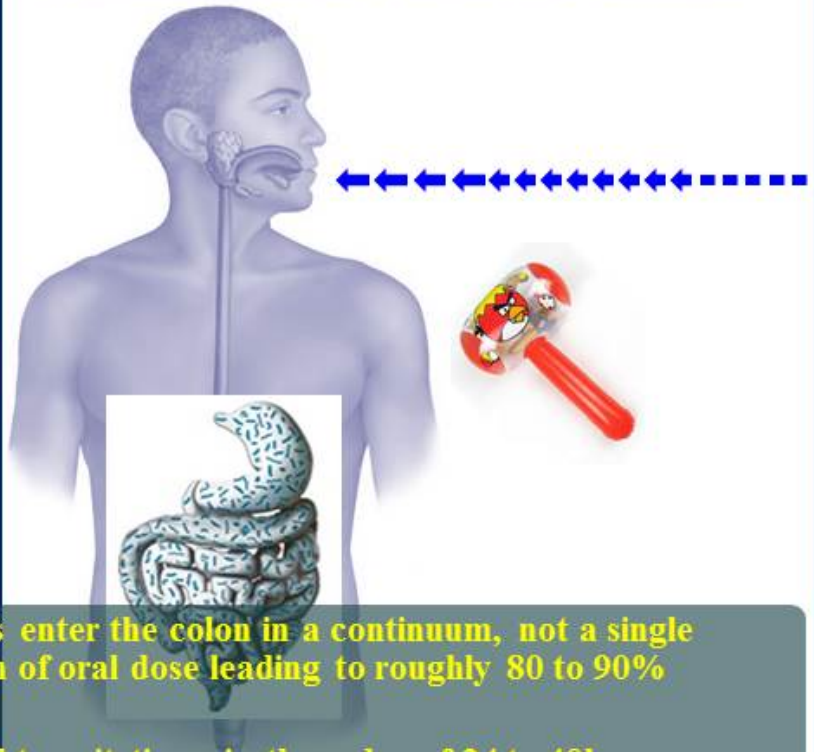
- After oral ingestion, chemical residues in food can reach the colon due to incomplete absorption, enterohepatic circulation, or secretion across the intestinal epithelial mucosa.
- The fraction of the chemical residue (oral dose) available to the microbiota can be greatly affected by dose and dosing frequency as well as the extent of binding to intestinal contents and metabolism.
- What is critical in delineating this comparison of residue "loading" is the observation that the components contained within a single meal do not enter the colon as a bolus dose.



Acute Exposure Chronic

Acute intake of xenobiotic residues would be a single exposure event wherein the dose is ingested as a one-meal time event and transits down the gastrointestinal tract into the colon that contains no comparable levels of ingested xenobiotic residue.

In chronic exposure of xenobiotic residues, there is an assumption of daily ingestion of the xenobiotics that is each day the ingested meal enters into an intestinal tract that already has xenobiotics spanning the intestine due to ingestion from the day before over a lifetime.



- **Collectively, studies show that ingested materials enter the colon in a continuum, not a single bolus, with colonic fill starting as early as 1 to 5h of oral dose leading to roughly 80 to 90% loading within 12h.**
- **Excretion also begins within 12h with mean total transit times in the order of 24 to 40h.**

Toxicity Tests

- Acute and Chronic Systemic Toxicity
- Carcinogenicity
- Dermal Penetration
- Ecotoxicity
- Endocrine Disruptors
- Genotoxicity
- Neurotoxicity
- Pharmacokinetics & Metabolism
- Phototoxicity
- Repeated Dose/Organ Toxicity
- Reproductive & Developmental Toxicity
- Allergenicity/Skin Sensitization
- Microbiome Toxicity and other Microbiological Effects

Methods for Measuring the Effects of Xenobiotic Compounds on the Human Intestinal Microbiota

In vitro

- Shorten term anaerobic incubation of fecal suspensions
- Continuous and semi-continuous culture systems
- Simulated gut models
- Intestinal fed batch culture
- Gut-on-a-Chip

Ex vivo

- Explant cultures (tissue cultures) extracted from the colon or rectum

In vivo

- Conventional and germ-free laboratory animals
- Human flora associated animals
- Human volunteers

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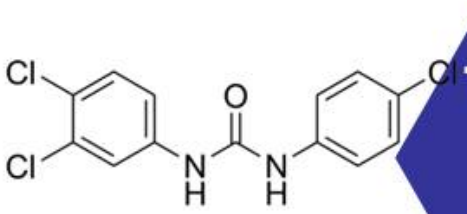
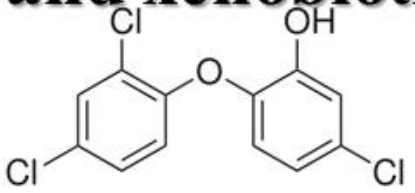
Project Number: E0220101 7/9/2015

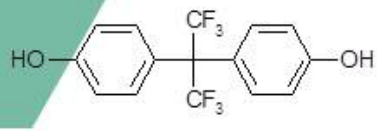
The human gut microbiome and xenobiotics



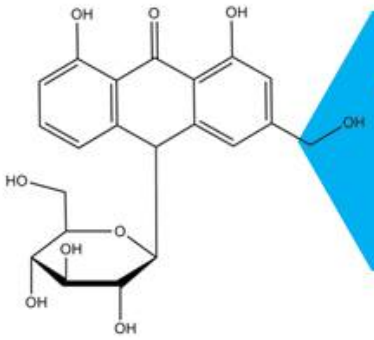
Triclocarban And Triclosan



Bisphenol AF



GIT Homeostasis



Aloin/ Aloe vera

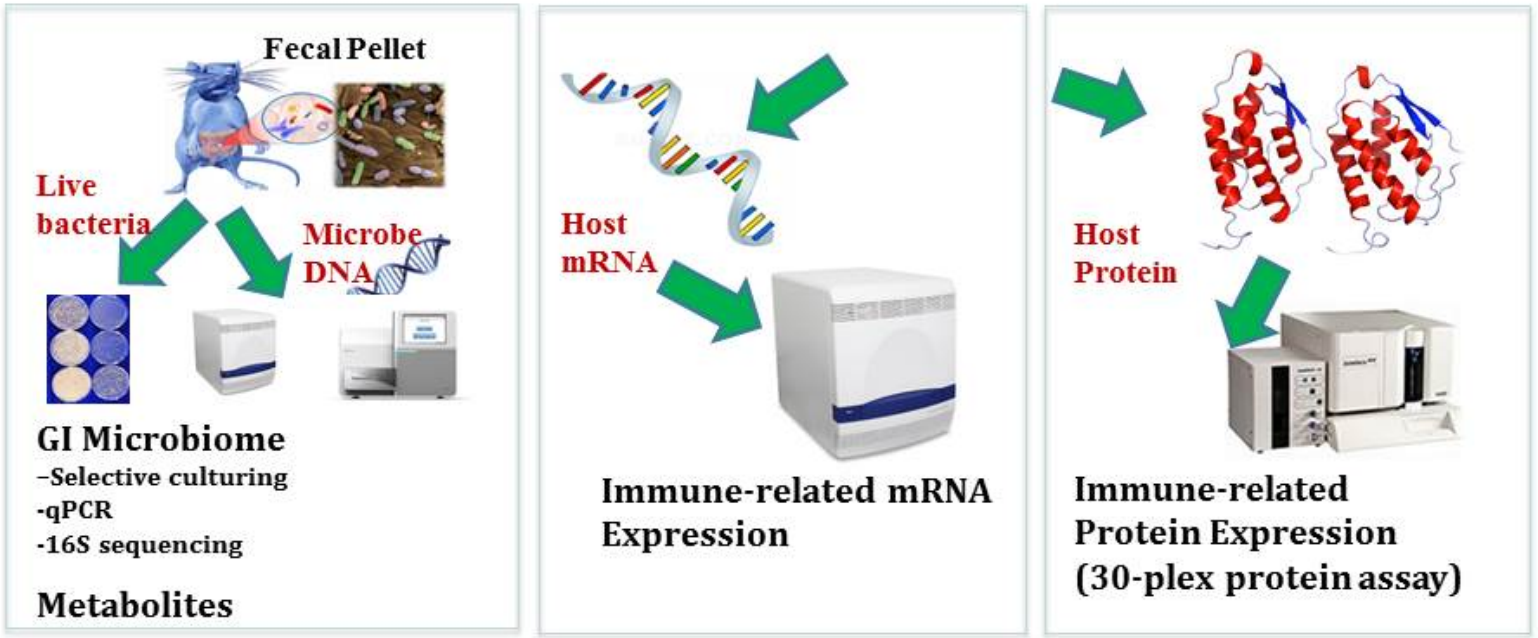
Arsenic

$_{33}As$

Silver

$_{47}Ag$

Intestinal Microbiome Analysis Approaches



Microbiome NTP Projects Overall Summary and Status

Xenobiotic Compounds	Experimental Model	Experiments	Status
Silver Nanoparticles	Rat	➢ Host Microbiome ➢ Immunotoxicity	✓ Completed
Arsenic	Mouse	➢ Fecal aerobic and anaerobic bacterial culture ➢ Intestinal Microbiome (16S) ➢ Immunotoxicity	✓ Completed o In progress o In progress
	Rat	➢ Fecal aerobic and anaerobic culture ➢ Intestinal Microbiome (16S) ➢ Immunotoxicity	✓ Completed; Data analysis ongoing o Samples Collected o Samples Collected
Aloin	In vitro	➢ MIC on pure *E.coli* and *Lactobacillus* ➢ Microbiome, SFCAs and Aloin metabolism in fecal content	✓ Completed o Ongoing
Bisphenol AF	Rat	➢ Intestinal Microbiome (16S) ➢ Immunotoxicity	o Samples Collected o Samples Collected
Triclosan/ Triclocarban	Rat	➢ Fecal aerobic and anaerobic bacterial culture ➢ Intestinal Microbiome (16S) ➢ Immunotoxicity	o Planning

Research Team and Acknowledgements

NCTR

- Dr. Carl E. Cerniglia (DM)
- Dr. Sangeeta Khare (DM)
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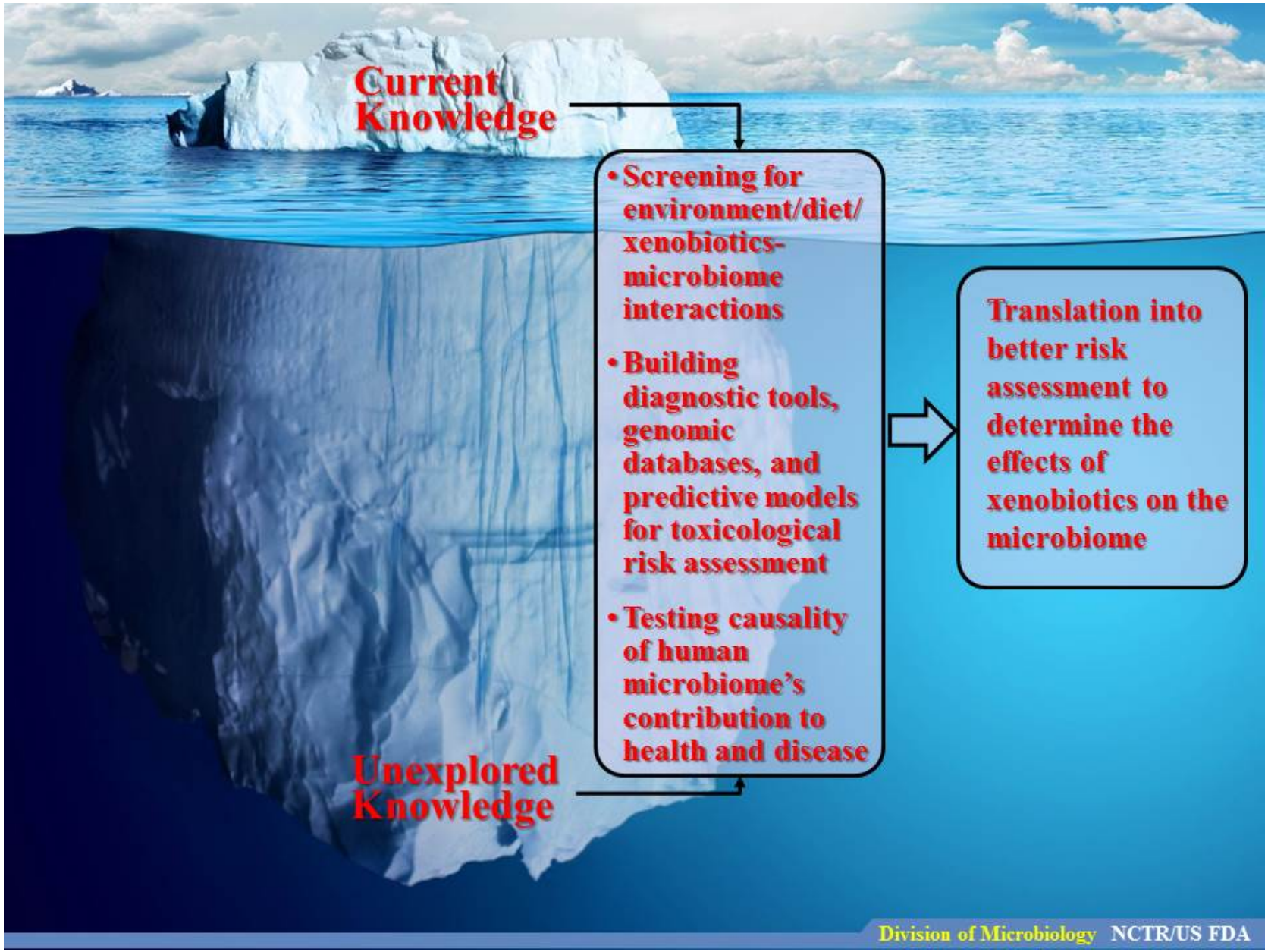
- Ms Kylie Krohmaly (DM)
- Dr. Paul Howard (OSC)
- Ms Michelle Vanlandingham (DBT)
- Dr. Dan Doerge (DBT)
- Dr. Mary Boudreau (DBT)

NTP/NIEHS

- Dr. Vicky Sutherland
- Dr. Nigel Walker


Current Knowledge
• Screening for environment/diet/ xenobiotics-microbiome interactions
• Building diagnostic tools, genomic databases, and predictive models for toxicological risk assessment
• Testing causality of human microbiome's contribution to health and disease
Translation into better risk assessment to determine the effects of xenobiotics on the microbiome
Unexplored Knowledge
Division of Microbiology NCTR/US FDA

Thanks a lot!!!

Food and Drug Administration
NCTR
National Center for Toxicological Research

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